JOTTINGS FROM THE BIOLOGICAL LABORATORY OF SYDNEY UNIVERSITY.

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14. On a remarkable Flat-Worm Parasitic in the Golden Frog.

(Plate xx.)

It is several years since my attention was first directed to a remarkable worm living as a parasite in the common gold-and-green frog *(Hyla aurea)*; but, though specimens have been met with several times since in the frogs dissected in the laboratory, I have rarely had the opportunity of examining them before they had been to some extent broken or destroyed. Altogether I think six or seven cases of frogs infested with the parasite in question have been met with during the last three years out of at least 150 specimens dissected; so that this parasite, though not uncommon, is by no means so general in its occurrence in this species of frog as *Rhabdonema*, *Distoma* or *Myxosporidium*, all of which are to be found in nearly every individual carefully examined for them.

The worm at present under consideration occurs sometimes in the subdermal lymph-sinuses—in some cases on the ventral, in others on the dorsal side—or it is found between the superficial muscles of the thigh or the leg, or (this I observed only in one instance) in the pleuro-peritoneal cavity.

It has the form and appearance of a long and narrow, transversely ribbed white ribbon. The longest specimen—which still had the appearance of being incomplete behind—was about two inches in length when moderately extended, and about a tenth of an inch in greatest breadth. Uninjured specimens showed incessant and active movements. These consisted of alternate elongations and contractions of the body which were most marked at the

anterior end; this extremity would be pushed out with great rapidity and the whole of the anterior part of the body would become stretched and narrow ending in front in a point. Then the anterior end would be drawn back and that part of the body greatly contracted and thickened—the extreme anterior end sometimes becoming involuted within the part immediately following it.

The body is divided into a number of narrow segments which are very sharply defined in front, except when the body is stretched to its utmost, but becomes less marked behind. These are not all perfectly regular, but their length is in general less than a third of the breadth of the body. A well-marked notch, most distinct when the animal is contracted, is found at the anterior end. On no part of the body is there any opening to be found, and there is no vestige of hooks or suckers.

At first I was greatly puzzled as to the relationships of this remarkable parasite, but an examination of sections showed at once that I had to do with one of the so-called "parenchymatous" worms; and the absence of an alimentary canal proved that it was among the Cestodes and not among the Trematodes that I had to look for its nearest relatives. That it must be a scolex was evident from the situation in which it was found, as well as from the absence of reproductive organs.

Only three genera of the Cestodes are known to have solid, elongated scolices, viz., *Tetrarhynchus*, *Schistocephalus* and *Ligula*. The movements of the anterior end of the scolex of *Tetrarhynchus gigas* from the muscles of *Orthagoriscus mola* as described by Van Beneden resemble closely those of the parasite of Hyla—"Cette partie du corps s'étend, se raccourcit, s'étrangle, se recourbe, tantôt à droite, tantôt à gauche, et semble chercher avec anxiété un tissu à perforer."* But the form is cylindrical, or nearly so, and there is an entire absence of segmentation.

* "Sur les vers parasites du poisson-lune *(Orthagoriscus mola)* et le *Cecrops Latreillii* qui vit sur ses branchies." Bulletin de l'Académie Royale de Belgique, Tome XXII.

The scolices of *Schistocephalus* and *Ligula*, then, would seem to be the nearest known allies of the parasite under examination. These are elongate, ribbon-like, segmented, and notched at the anterior end. They are found in certain special situations in the body-cavity of Cyprinoid fishes, and, unlike most scolices, when ready for their final transformation into the mature tape-worm, perforate the wall of the body of their host by their own efforts, and pass out into the water, whence they are taken up into the alimentary canal of some water-bird, in which they complete their development. Before proceeding with the comparison of the parasite of the *Hyla* with *Ligula* and *Schistocephalus* I shall give a detailed account of my observations on the minute structure of the former, as some of these are of importance as bearing on the question of affinities.

The whole surface is covered with a chitinous cuticle (fig. 3, *cut*), which consists of two layers, an outer thinner and an inner thicker. The outer layer is a homogeneous membrane in the anterior part of the body of my best-preserved specimen, while further back it is divided by numerous vertical fissures, so that in section it has the appearance of being composed of numerous close-set papillæ. The inner thicker layer is perfectly homogeneous. Beneath it is a thin layer of minute highly refracting bodies *(x)*, which are apparently nothing else than thickened and specialised outer ends of the fibres of the parenchyma muscle, which are found to be continuous with them; and these bodies seem to be attached to an exceedingly thin continuous layer of similar appearance which immediately underlies the cuticle. The fibres of the parenchyma muscle would thus appear to be inserted not directly into the cuticle, but into a thin membrane—perhaps of elastic character—immediately underlying the latter.

At a little distance below this is an irregular layer *(ep)*, of tolerably large nuclei, each having around it a small quantity of protoplasm which sends out slender fibre-like processes inwards and outwards. This layer doubtless represents an epithelium of vertically elongated cells of which the bodies are imperfectly developed or have become attenuated.

The greater part of the interior is in most parts of the body occupied by the form of connective tissue characteristic of Flatworms and commonly called parenchyma—a faintly granular uncolourable matrix having running through it felt-like bundles of very delicate fibres with stellate, angular or rounded nuclei here and there. Occasional cavities and channels occur in this ground-tissue. Embedded in it, especially in its outer part, are great numbers of the *calcareous corpuscles (cal)*, so characteristic of Cestodes. These are rounded bodies which for the most part show a distinction into a central nucleus and an outer rind. The central part consists of a (usually) rounded body of strongly refracting appearance and perfectly homogeneous; while the outer part, which does not refract the light so strongly, is composed of several concentric layers. These bodies dissolve and nearly or completely disappear under the action of strong acids, but without effervescence. Caustic potash only renders the outer part clearer. From their appearance when acted on by dilute acid, it would appear that the two parts of the corpuscle are of different composition—the inner part becoming before it is entirely dissolved converted into a cluster of granules while the outer part never becomes granular, but only gradually becomes less and less distinct till a fine outline alone remains.

Separated from the epidermal layer by an interval of parenchyma with calcareous corpuscles is a zone of longitudinally-arranged muscular fibres *(l.m.)*, divided into parallel bundles by intervals of parenchyma and parenchyma muscle. These are slender, elongated, nucleated fibres. A transverse or circular layer of muscle is entirely wanting.

Running through the parenchyma are numerous bundles of the very slender muscular fibres of the parenchyma *(p.m.)*. Most of those are nearly at right angles to the surface, though many are oblique; as they approach the surface the fibres separate out from one another and divide to become attached in the manner already described.

Though a nervous system can be detected, it is very indistinct. In the head portion of the body a number of fibres having exactly

the appearance of the nerve fibres of Trematodes and Cestodes run forwards into a central mass of tissue having numerous particles of pigment embedded in it. Though this, from its relation to the nerve-fibres, is probably of the nature of a ganglion, it is scarcely distinguishable from the surrounding parenchyma, and does not contain a single cell that could be set down as a ganglion-cell. I did not succeed in tracing any nerve cords in the hinder part of the body.

The only internal organs that are well developed are the system of canals. A main trunk of considerable size runs along each side. This frequently bifurcates, so that two main canals may run parallel with one another through several segments—sometimes again joining to form one, or one of them breaking up into branches—the other continuing on as the main canal. The branches given off are numerous and these again divide and subdivide; the giving off of the branches has no regular relationship to the segments. The main trunks do not extend quite to the head end; their posterior terminations, in the absence of complete specimens, were not made out.

On comparing these observations with what I can find published regarding *Ligula* I observe many points of agreement. The general shape of the body—elongate and ribbon-like with somewhat irregular segmentation—is the same in both cases. The form of the head end is somewhat different apparently, and the dorsal and ventral sucking grooves present in *Ligula* are absent in the parasite of Hyla.

The account of the minute structure of *Ligula* given by Donnadieu* which is the only one, so far as I can ascertain, published of recent years, leaves a great deal to be desired, and has evidently not been drawn up from good preparations. The cuticle (epiderme), he describes as in several layers (*three* are represented in the figures) which increase in thickness from without inwards, which are perfectly homogeneous. His representation

* "Contributions à l'histoire de la Ligule;" 'Journal de l'anatomie et de la physiologie,' tome 13, 1877. The same remark applies to Duchamp's "Recherches sur les Ligules" (Paris, 1876).

of the rest of the minute structure is so totally different from what we find in other Cestodes that I think it cannot be correct; and there would be little advantage in attempting a detailed comparison. The mode of termination of the principal trunks of the canal system by opening in front in the neighbourhood of the sucking-grooves would seem to constitute an important difference between Ligula as described by Donnadieu and the frog-parasite; the presence in the former of distinct though immature reproductive organs is also an important distinguishing feature.

The histology—as regards the cuticle, subcuticular cell-layer, muscular fibres and "parenchyma"—is very similar to that of such mature *Tæniæ* as I have studied (*T. expansa* of the sheep, *T. crassicollis* of the cat, *Dibothrium microcephalum* of the sun-fish and *T.* sp., of the emu); but the arrangement of some of these parts is slightly different. Thus the layer of circular muscular fibres, which is well developed in mature *Tæniæ*, is entirely absent here in the scolex from *Hyla aurea*, and the longitudinal fibres do not form the complete layer which they present in the former.

The entire structure of the parasite indicates unmistakably its Cestode nature; and superficial resemblances, at least, indicate a relationship with *Ligula;* but what comparisons I have been able to make between the two former as regards internal structure seem to point to considerable differences. The discovery of the mature worm can alone set at rest completely the question of the affinities of this remarkable parasite.

EXPLANATION OF PLATE.

Fig. 1.—Anterior portion of the Cestode parasite of *Hyla aurea*, magnified. From a preserved specimen.

Fig. 2.—Portion of a transverse section. × 100.

Fig. 3.—A portion of a similar section. × 800. *cut*, two-layered cuticle. *ep*, 'epidermis.' *p.m.*, parenchyma muscle. *x*, layer in which the fibres end. *l.m.*, longitudinal muscular fibres. *cal*, calcareous corpuscles.